EFFECTS OF pH AND IONIC STRENGTH ON ALUMINUM TOXICITY TO EARLY DEVELOPMENTAL STAGES OF RAINBOW TROUT (Salmo gairdneri Richardson)

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ABSTRACT

Laboratory studies were conducted to examine the effects of inorganic aluminum (0.02 to 1.0 mg L^{-1}) in water ranging in pH (4.0 to 7.2) and ionic strength (0.73 to 5.43 mmoles L^{-1}) on the survival of early developmental stages of rainbow trout (*Salmo gairdneri*). Aluminum toxicity increased with decreasing pH and ionic strength. Trout became more tolerant to hydrogen ion with progressive level of development (cleavage egg < eyed egg << yolk sac fry ≃ swim up fry) while sensitivity to aluminum increased with advancing developmental stages. All stages tested were unaffected by exposure to low pH (4.5) and aluminum (<0.02 mg L^{-1}) with the exception of the cleavage egg stage (5 day old embryo). Nominal total aluminum concentrations of ≥0.5 mg L^{-1} at pH 5.5 and ≥0.1 mg L^{-1} at pH 4.5, were acutely toxic to yolk sac and swim up fry stages. Cleavage eggs demonstrated the greatest tolerance to aluminum at pH levels of 7.2 to 4.5, being unaffected by concentrations up to and including 1.0 mg L^{-1}. The presence of aluminum was beneficial to the survival of cleavage embryos at pH 4.5, reducing mortality from 27.5% at low aluminum (<0.02 mg L^{-1}) to 4.5% at high concentrations (1.0 mg L^{-1}).

Reductions in whole body content levels of Na^+, Cl^- and to a lesser extent K^+, Ca^{++} and Mg^{++}, were observed in alevin rainbow trout exposed to 0.4 mg L^{-1} aluminum at low pH (5.5-5.0). Salt losses were not observed in this pH range at low aluminum concentration (<0.02 mg L^{-1}).

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INTRODUCTION

Much of the information concerning the lethal and sublethal effects of low pH on fish was generated to assess the effects of acid mine wastes (Lloyd and Jordan, 1964; Packer and Dunson, 1970; Menendez, 1976). More recently, hydrogen ion (H^+) toxicity, has been linked to the disappearance or decline of fish populations via the more subtle process of environmental acidification (Jensen and Snekvik, 1972; Beamish et al., 1975; Beamish, 1976; Leivestad and Muniz, 1976). Impairment of reproduction (Almer et al., 1974) through inhibition of gonadal maturation (Beamish et al., 1975; Lockhart and Lutz, 1977), impairment of embryogenesis, oogenesis and fertilization (Ruby et al., 1977, Craig and Baksi, 1977) and egg and larval mortalities (Jensen and Snekvik, 1972; Leivestad et al., 1976) appear to be important mechanisms of recruitment failure that have contributed to fisheries decline. Levels of pH however, that have been reported to be lethal to fish in acidified waters in the field, are generally much higher (pH 5.0 to 6.0) than lethal pH levels determined under controlled laboratory conditions (Daye and Garside, 1975, 1977). Differences between laboratory and field estimates of hydrogen ion toxicity have suggested that additional stress factors must be involved in the field.

Short term spatial and temporal changes in water quality have been shown to account for some of the observed differences in fish response. Marked increases in H^+ concentrations have been observed during spring and fall runoff periods (Beamish and Harvey, 1972; Lockhart and Lutz, 1977; Jeffries et al., 1979; Scheider et al., 1979) and have caused death in fish (Schofield, 1976; Leivestad and Muniz, 1976; Grande and Andersen, 1979). The decreased pH of precipitation due to strong acids (H_2SO_4; HNO_3) has resulted in the increased mobilization of metals, in particular aluminum, from soils (Almer et al., 1978; Johnson, 1979). This has resulted in changes in both the concentration and forms (Driscoll et al., 1980) of aluminum that have been observed in low alkalinity, ionic strength waters in parts of New York State (Cronan and

Schofield, 1979), Norway (Wright et al. 1976; Wright and Henriksen, 1978), Nova Scotia (Watt, 1980 in Farmer et al., 1980) and Ontario (Scheider et al., 1979). Observations by Cronan and Schofield (1979), indicated that higher concentrations of aluminum in acid stressed waters could be detrimental to fish survival.

The importance of short term, abrupt increases in aluminum and hydrogen ion concentrations on fish, particularly during the early stages of development, is poorly understood. Laboratory studies with brook trout (Salvelinus fontinalis) and white sucker (Catostomus commersoni) have demonstrated differences in species and life stage sensitivities to aluminum (Baker and Schofield, 1982). The early fry stages were more sensitive to aluminum than eggs. However, the study did not include a comparison between early and late egg stages, which have been shown to differ in their relative sensitivities to pH (Daye and Garside, 1979). Aluminum was found to be acutely toxic to brook trout fry in low pH (4.2 to 5.6) and calcium (2.0 mg L^{-1}) waters at concentrations as low as 0.2 mg L^{-1} (Cronan and Schofield, 1979; Baker and Schofield, 1982). Chemical characterization of aluminum identified the labile monomeric inorganic fraction as the most toxic form (Driscoll et al, 1980). Acute toxic effects of aluminum were not observed at concentrations up to 200 mg L^{-1} and pH levels of 6.0 to 7.0 in laboratory tests with rainbow trout. However, the tests were conducted with older fry stages and under different water quality conditions (Freeman and Everhart, 1971; Hunter et al., 1980). Although toxic effects of aluminum have primarily been associated with dilute acid waters, relatively little is known about the acute effects of aluminum in waters of higher pH and ionic concentrations.

The present laboratory studies were designed to identify critical periods of fish development during egg and fry stages that would be affected by short term exposure to elevated concentrations of aluminum and hydrogen ion. Rainbow trout were exposed for 8 days during the cleavage egg, eyed egg, yolk sac fry and swim up fry stages to aluminum (<0.02 to 1.0 mg L^{-1}) at different pH levels (4.5 to 7.2). A 12 day recovery period was provided following the test exposure to identify latent toxicity. A second series of experiments was conducted with trout fry, to examine the acute toxic effects of aluminum and pH in low, intermediate and high ionic strength water, selected to approximate a range of natural lake water chemistries.

MATERIALS AND METHODS

Fish Stocks

Newly fertilized eggs of rainbow trout (*Salmo gairdneri* Richardson) were obtained from two local certified disease-free fish hatcheries. The eggs were reared to the swim-up fry stage in vertical flow incubation chambers, supplied with $10^{o}C$ dechlorinated Lake Ontario tap water at a rate of 13 to 16 L min^{-1}. The eggs were inspected periodically and dead eggs were removed to minimize fungal growth. Rates of development and percent mortalities of trout reared to swim-up, were similar for the different stock populations.

Dilution Waters

The experiments were conducted in artificially softened Lake Ontario tap water containing total ionic strength levels as follows: high (5.43 mmoles L^{-1}), intermediate (2.76 mmoles L^{-1}) and low (0.73 mmoles L^{-1}) (Appendix 1). The lower ionic strength waters were obtained by dilution of the dechlorinated tap water with appropriate amounts of reverse osmosis-treated tap water.

The pH of the test solutions was ajusted 48 hours prior to the addition of aluminum using 1.0 N sulphuric acid (H_2SO_4), to allow pH stabilization and removal of excess free CO_2. Nominal total aluminum concentrations were achieved by addition of aluminum from stock solutions of analytical grade aluminum sulphate [$Al_2 (SO_4)_3 .18 H_2O$] and waters were aged for 24 hours prior to the addition of the fish. The pH values were checked (Radiometer pHM61) three times daily thereafter and adjusted to ±0.1 of the desired pH level using 0.1 N H_2SO_4. Measured pH levels rarely exceeded 0.2 units of nominal test levels.

The control and test solutions were aerated at a rate of 5-10 ml L^{-1} min^{-1} to provide continuous mixing and to maintain dissolved

oxygen levels near saturation. The experiments were conducted at 10± $1^{o}C$. Light intensity 10 cm above the water surface, ranged from 10-20 lux during a 14-hour light period.

Water samples were withdrawn from all solutions after 0 and 96 hours of testing for determinations of pH, alkalinity, $SO_4^=$ and total unfiltered aluminum. Water hardness, major ions (Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, F^-), DOC, DIC and trace metals (Cd, Cr, Cu, Co, Ni, Mn, Zn, Pb) were measured at the beginning of each test and after renewal of the test solutions. Chemical analyses were performed by the Ontario Ministry of the Environment, Laboratory Services Branch, according to the procedures described in Outlines of Analytical Methods (1981).

The relative concentrations of aqueous inorganic aluminum species (i.e. hydroxides, fluorides, sulphates) were calculated from nominal aluminum concentrations, measured water chemistry parameters and thermodynamic constants, derived for aluminum in equilibrium with micro-crystalline gibbsite (pers. comm. Bruce Lazerte, University of Guelph, Ontario/Ontario Ministry of the Environment).

Concentrations of dissolved organic carbon in the test waters were low (0.4 to 1.6 mg L^{-1}). Preliminary determinations of the relative amounts of inorganic and organic aluminum in the test waters indicated that all or most of the aluminum existed as either suspended or dissolved inorganic species.

Decreases in measured total aluminum concentrations in the test waters were observed over the 96 hour period and varied with the pH and ionic strength of the solutions (Appendix II). In the test waters, pH 6.0 to 7.2, measured aluminum concentrations in the low ionic strength water (0.7 mmoles L^{-1}) were as low as 20% of nominal concentrations. In the intermediate and high ionic strength waters (2.8 and 5.4 mmoles L^{-1}) measured aluminum concentrations were within 20% and 5% of nominal concentrations respectively. Measured aluminum concentrations in these waters however, were greater than or equal to the respective theoretical total dissolved aluminum concentrations (i.e. over saturated Al solutions). One exception was noted at 0.5 mg L^{-1} aluminum, pH 7.2 in the low ionic strength water. Here, measured aluminum after 96 hours was

equal to one half of the theoretical dissolved aluminum. In the acid solutions (pH <5.5) measured aluminum concentrations were as low as 40 to 50% of nominal concentration. At low ionic strength, solutions remained oversaturated with respect to the theoretical dissolved aluminum while at the higher strength levels solutions were up to 50% undersaturated.

Life Stage Sensitivity Comparison

Four successive early developmental stages of rainbow trout, were selected for comparison of their relative sensitivities to aluminum and pH in the low ionic strength water (0.73 mmoles L^{-1}). Exposures of the cleavage embryo, eyed embryo, yolk sac fry and swim up fry stages were initiated 1, 17, 30 and 42 days after fertilization, respectively (Fig. 1). The experiments were divided into three exposure periods: acclimation, test and recovery. Eggs and fry were held in the test water under control conditions (Al = 0.02 mg L^{-1}; pH = 7.2) for five days prior to testing. Each stage was then exposed for eight days, to nominal aluminum concentrations of <0.02 (control), 0.1, 0.5 and 1.0 mg L^{-1} at each of four pH levels (4.5, 5.5, 6.5, 7.2). The 0.1 mg L^{-1} aluminum concentration was not included in the concentration series conducted at pH 7.2. The tests were conducted under static conditions with 100% renewal of the test solution after four days. Eggs and fry that remained alive at the end of the test were returned to "control" conditions and held for an additional twelve days.

All developmental stages were tested in modified embryo-larval rearing chambers (Shafland, 1979). The exposure chambers (Fig. 2) were designed to retain a sufficient volume of water (50 ml) when removed from the test solution in order to minimize handling stress during transfer. Two chambers, each containing 35 organisms were immersed in 10 L of test solution in each of two, 25 L cylindrical plastic tanks.

The criteria for death were discolouration of the egg membrane and cessation of the heart in the post-hatching fry stages. Discoloured cleavage eggs were cleared in 10% acetic acid to differentiate between fertilized and non-fertilized eggs.

Fig. 1 EMBRYO/ALEVIN EXPOSURE SEQUENCE

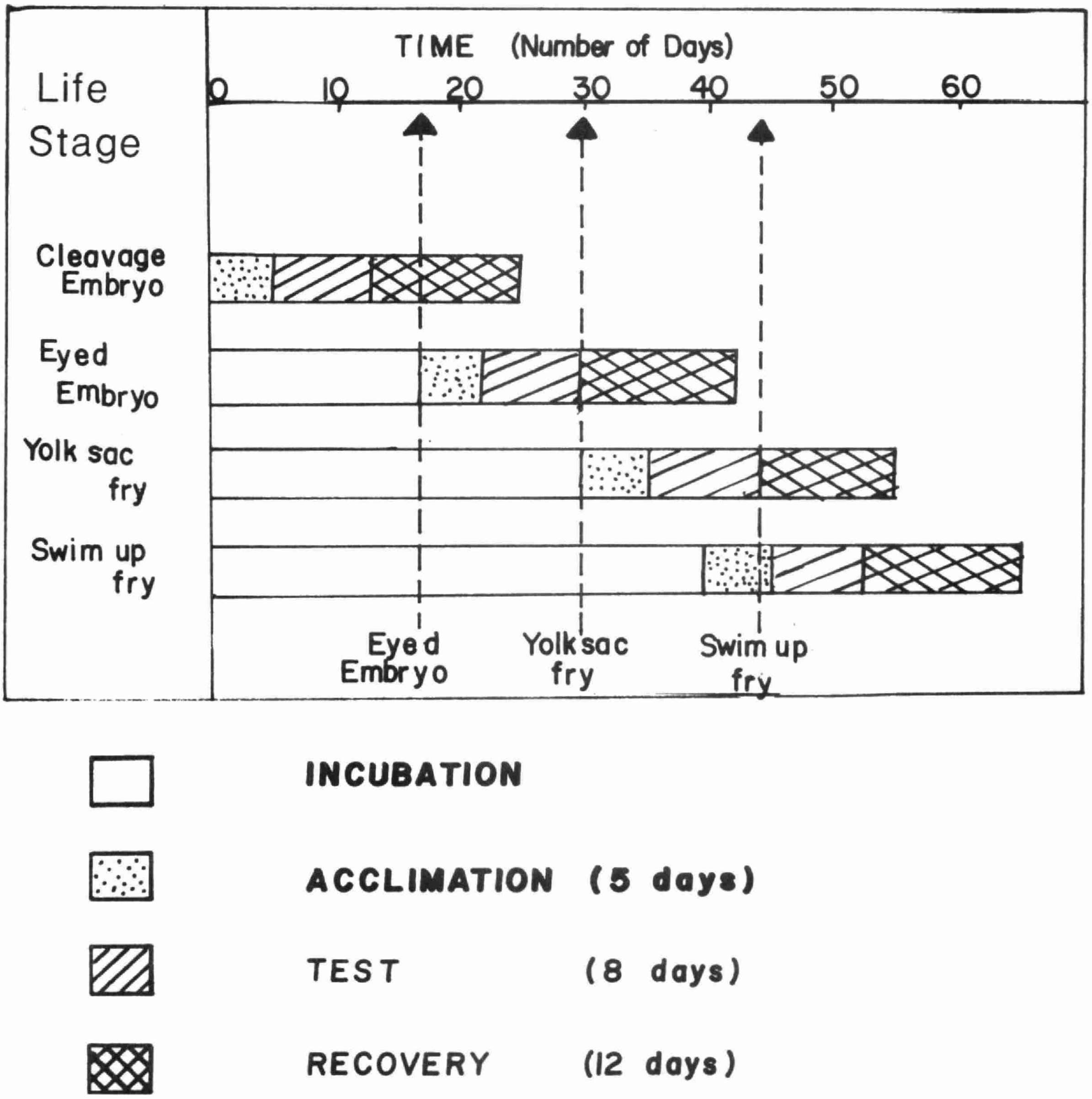
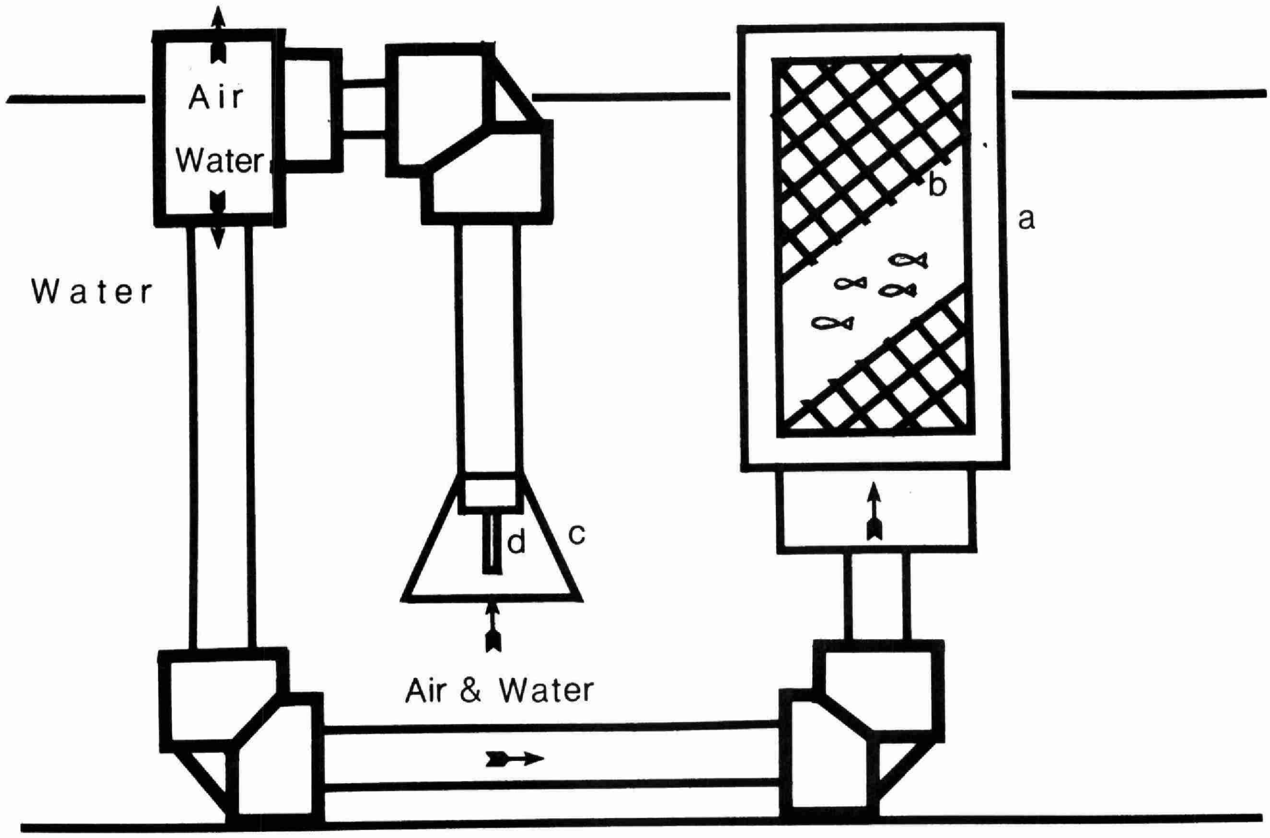


Fig. 2 System Used For Exposing Egg and Fry Stages of Rainbow Trout to Test Conditions



(a) Plastic Test Chamber (c) Funnel

(b) Nylon Screen (d) Airline

Ionic Strength Effects

(1) Acute Lethality

96-hour static bioassays were conducted to compare the effects of pH and ionic strength on aluminum toxicity to alevin rainbow trout. Alevins ranging from 0.5 to 2.0 gm were subjected to aluminum (0.02 to 20 mg L^{-1}) in waters of different ionic strength (0.7, 2.8, 5.4 mmoles L^{-1}) and pH (4.0, 4.5, 5.0, 5.5, 6.0). The fish were held in each of the respective test waters for 60 days prior to the adjustment of pH or aluminum. During this period the fish were fed EWOS trout feed at a rate of 0.4% of total body weight per day. Feeding was discontinued 24 hours prior to the test. Fish removed for testing were starved during the 96 hour exposure period.

Ten alevins were placed into each of two, 25 L plastic tanks containing 20 L of test solution. Mortality was recorded three times daily and final percent mortality was determined after 96 hours.

(2) Determination of Electrolyte Levels.

Whole body electrolyte levels were determined in alevin rainbow trout subjected to control (0.02 mg L^{-1}) and high (0.4 mg L^{-1}) aluminum at pH levels of 5.0 to 6.0 in each of the low, intermediate and high ionic strength test waters. The tests were carried out in duplicate with ten fish per container. Acclimation of the fish to the respective test waters and feeding prior to the test were similar to the experiments described under 'Acute Lethality'.

Electrolyte levels ($\bar{x}$ ±S.D.) were determined from three, two-fish composite samples and were reported as mmoles $100g^{-1}$ wet weight. Fish subjected to the 0.4 mg L^{-1} aluminum treatment at pH 5.5 and 5.0 were removed for analysis prior to death (after 48 hrs.) from the low and intermediate ionic strength waters. Fish from the remaining treatments were removed at the end of the 96 hr. period.

Body Na^{+}, K^{+}, Ca^{++} and Mg^{++} levels were measured in fish exposed for 96 hours at pH 6.0, 5.5 and 5.0; body Cl^{-} levels

were measured in fish tested at pH 5.5 only. Cation levels were determined by atomic absorption spectrophotometry and Cl^- levels were analyzed by ion chromatography (Laboratory Services Branch, Ontario Ministry of the Environment).

Whole body electrolytes except for Mg^{++}, determined in alevins acclimated to the low and intermediate ionic strength waters were not significantly different ($p > 0.05$) from levels determined in fish reared from fertilization in the high ionic strength water (Appendix III).

Data Analysis

Percent mortality data of egg and fry stages exposed to different pH and aluminum treatments were subjected to arcsine transformation and compared for differences using one way analysis of variance. Estimates of the 96-hour median lethal concentration (LC50), median survival time (MST) and upper and lower fiducial limits were calculated by the log probit analysis (least squares method, Hubert, 1980). The LC50 and MST values determined for aluminum in waters of different pH and ionic strength were compared for significance by the standard error of the difference (Sprague and Fogels, 1977). Mean values for whole body Na^+, Cl^-, Ca^{++}, Mg^{++} and K^+ concentrations in alevins were compared for differences using one way analysis of variance with pairwise comparisons of the treatment means according to the Scheffe's S method.

RESULTS

Life Stage Sensitivities to Aluminum/pH

Rainbow trout became more tolerant to hydrogen ion with progressive level of development (cleavage egg < eyed egg << yolk sac fry ≈ swim up fry) while sensitivity to aluminum increased with advancing developmental stages. Survival and development of the cleavage egg (five day old

embryos), yolk sac and swim up fry stages were unaffected after eight days exposure to total nominal aluminum concentrations as high as 1.0 mg L^{-1} at pH 7.2 and 6.5 (Fig. 3). Eyed eggs were moderately sensitive to aluminum at these pH levels, experiencing 14.2 to 21.6% mortality at high aluminum (1.0 mg L^{-1}). All stages with the exception of cleavage embryos, were able to tolerate an 8 day exposure to low pH (5.5 and 4.5) and aluminum concentration (<0.02 mg L^{-1}). Percent mortality of cleavage embryos tested at pH 4.5 decreased with increasing concentrations of aluminum, from 27.5% at low aluminum (<0.02 mg L^{-1}) to 4.5% at high aluminum (1.0 mg L^{-1}). For all other stages tested, aluminum toxicity increased with decreasing pH. Post-hatching fry stages demonstrated the greatest sensitivity to aluminum at low pH. Percent mortality of yolk sac and swim up fry, exceeded 90% at aluminum concentrations of $\geq$0.5 mg L^{-1} at pH 5.5 and $\geq$0.1 mg L^{-1} at pH 4.5. Eyed eggs were less sensitive to aluminum (1.0 mg L^{-1}) than fry, with mortalities ranging from 31.9 to 43.3% at pH levels of 5.5 and 4.5, respectively.

Eyed trout eggs experienced enhanced hatching rate in the lower pH waters (4.5 to 6.5) relative to the near neutral (pH 7.2) water for all aluminum concentrations tested during the 8 day test period (Fig. 4). Maximum hatch rate occurred at pH 5.5. Rate of hatch appeared to be little affected by aluminum concentrations up to and including 0.5 mg L^{-1}. However, delayed hatching of eyed eggs was apparent at high aluminum concentrations (1.0 mg L^{-1}). Mortalities continued to occur during the recovery period and were significant ($p < .05$) at all pH aluminum treatment levels relative to the control (pH 7.2/<0.02 mg L^{-1} Al Table 1). Latent toxic effects of either pH or aluminum were not evident for the other stages tested.

Ionic Strength Effects

(1) Acute Lethality

The acute effects of aluminum exposure on alevin rainbow trout tested in waters of different pH (4.5 to 6.0) and ionic strength

Fig. 3 RELATIVE SENSITIVITIES OF THE CLEAVAGE EGG, EYED EGG, YOLK SAC FRY AND SWIM UP FRY STAGES OF RAINBOW TROUT TO ALUMINUM AT DIFFERENT pH LEVELS

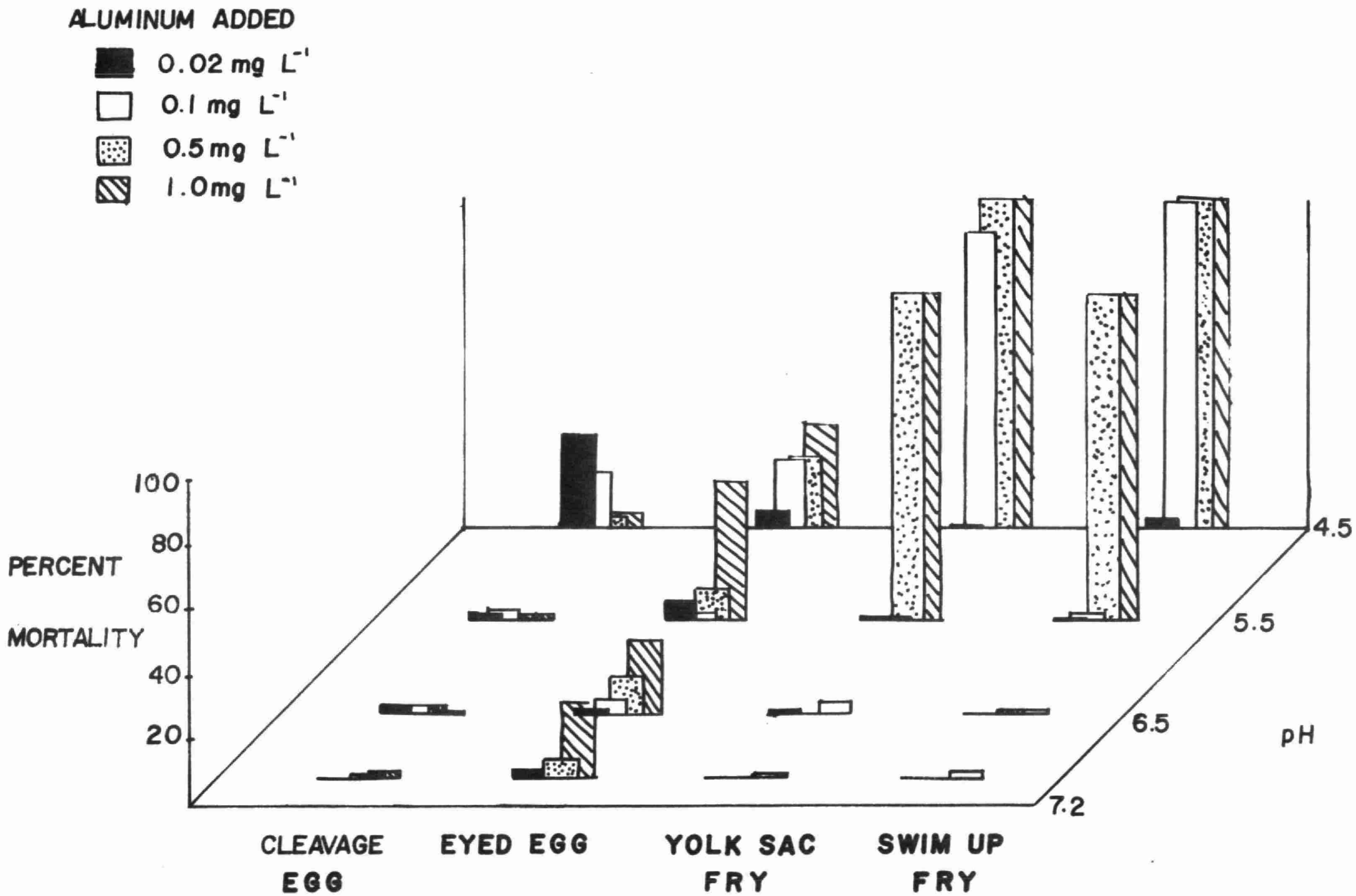
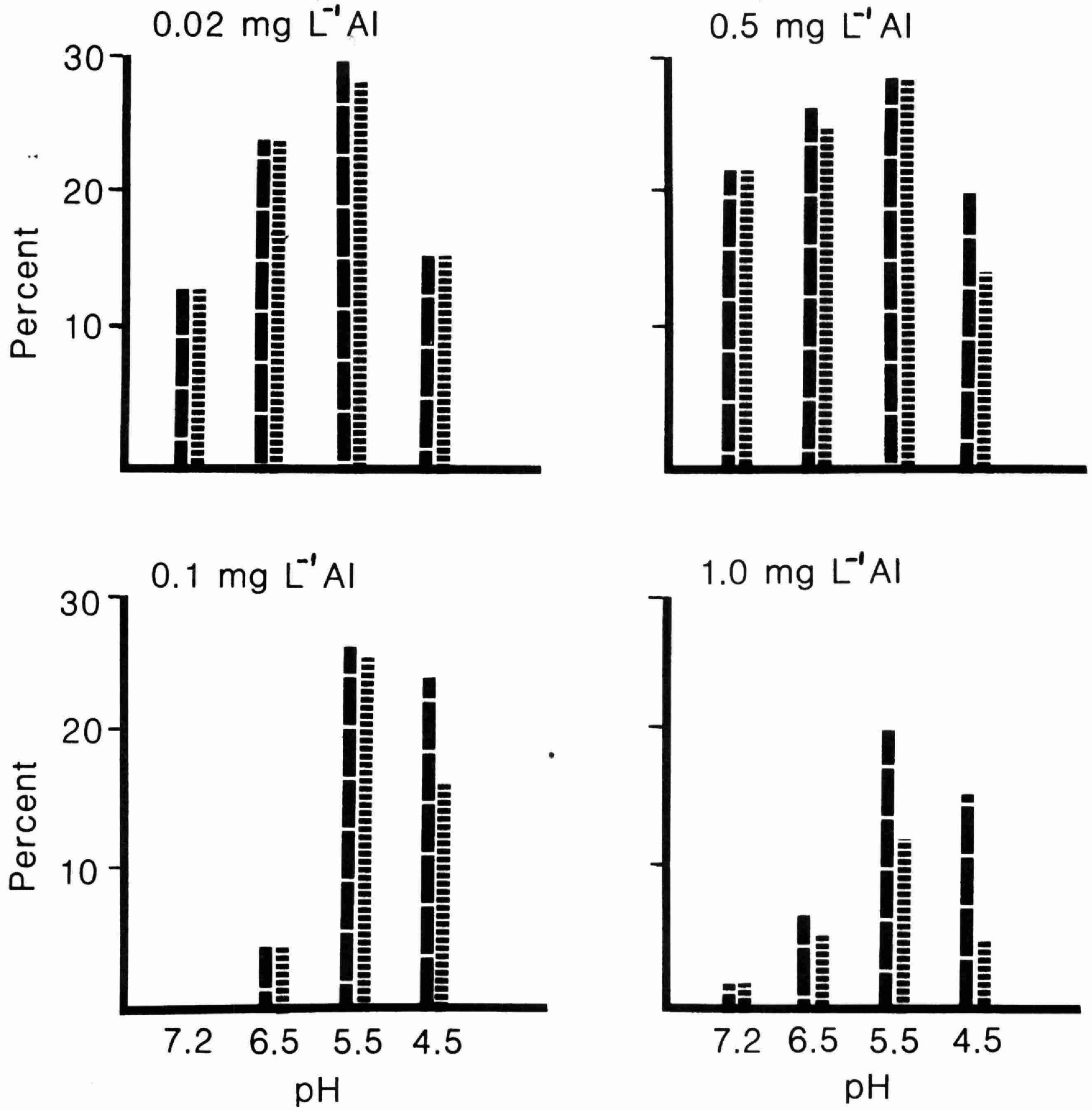


Fig. 4 Percentage Hatch of Trout Subjected for 8 Days to Aluminum at Different pH Levels During the Eyed Egg Stage



% Hatch during the 8-day test period

% Live Hatched Embryos of total eggs exposed during the 8-day test period.

Table 1: Mortality ($\bar{x}$ and 95% C.I.) of eyed embryos subjected to pH and aluminum after test (8 days) and recovery (12 days) periods.

pH	Aluminum Added ($mg\ L^{-1}$)	N	Cumulative Percent Mortality During Test $\bar{x}$ (95% C.I.)	Recovery $\bar{x}$ (95% C.I.)
7.2	<0.02	137	2.9 (0.7- 7.2)	4.5 (1.6- 9.1)
	0.05	138	5.8 (2.5-11.1)	56.0(47.1-64.2)
	1.0	141	14.5 (6.6-17.8)	52.6(43.0-60.9)
6.5	<0.02	140	1.4 (0.2- 5.0)	11.3 (6.7-17.9)
	0.1	140	4.3 (1.6- 8.9)	15.5(10.1-22.8)
	0.5	140	10.7 (6.1-17.1)	49.4(40.7-57.9)
	1.0	139	21.6(15.1-29.4)	46.4(37.6-54.7)
5.5	<0.02	138	5.8 (2.5-11.1)	30.9(23.6-39.6)
	0.1	138	2.2 (0.4- 6.2)	20.9(14.5-28.8)
	0.5	140	10.0 (5.6-16.2)	35.5(27.8-44.2)
	1.0	141	43.4(34.9-51.9)	84.8(78.1-90.5)
4.5	<0.02	140	5.7 (2.5-10.9	27.4(20.0-35.3)
	0.1	135	21.5(14.9-29.4)	33.3(25.5-42.0)
	0.5	141	22.7(16.0-30.5)	49.8(41.1-58.2)
	1.0	138	31.9(24.2-40.3)	67.1(58.9-75.1)

(0.7 to 5.4 mmoles L^{-1}) are summarized in Fig. 5. Aluminum toxicity increased with decreasing pH. At pH 6.0 nominal total aluminum concentrations as high as 1.0, 8.7 and 18.0 mg L^{-1} were tolerated for up to 96 hours at the low, intermediate and high ionic strength levels, respectively. Increases in the concentration of aluminum in these solutions were accompanied by a reduction in pH below 6.0 which resulted in 100% mortality of the fish, usually within 48 hours. At pH levels of 5.0 and 4.5, aluminum concentrations as high as 0.4 mg L^{-1} were non-lethal to trout at high ionic strength and were only moderately toxic at the intermediate level (Table 2). At low ionic strength the addition of 0.4 mg L^{-1} Al was acutely toxic to alevins at all pH levels below 6.0. The 96-hr LC50 values for aluminum (nominal) determined at pH 5.5, 5.0 and 4.5 were 0.31, 0.16 and 0.12 mg L^{-1}, respectively.

Mortality of trout exposed to pH 4.5 and control aluminum (<0.02 mg L^{-1}) conditions in the low, intermediate and high ionic strength waters ranged from 10 to 15%. Complete mortality of trout was observed at pH 4.0. The presence of aluminum at this pH had no observable effect on fish survival times. Fish survived longer in the higher ionic strength water (Fig. 6), but the differences were not significant ($p > 0.05$).

Aluminum toxicity increased in waters of decreasing ionic strength. Significant reductions in aluminum toxicity ($p < 0.05$) were observed at both the intermediate and high ionic strength relative to the low ionic strength water, at pH levels of 6.0 to 4.5. All estimated LC50 values, except for those determined at pH 6.0 however, were within an order of magnitude. Increasing ionic strength from low to intermediate and low to high concentration resulted in 3 and 4-fold decreases in aluminum toxicity respectively at pH levels of ≤5.5. The same relative change in ionic strength levels tested at pH 6.0, resulted in respective 8 and 18-fold decreases in aluminum toxicity.

Fig. 5 Lethal Concentrations of Aluminum to Rainbow Trout in Solutions Varying in pH and Ionic Strength

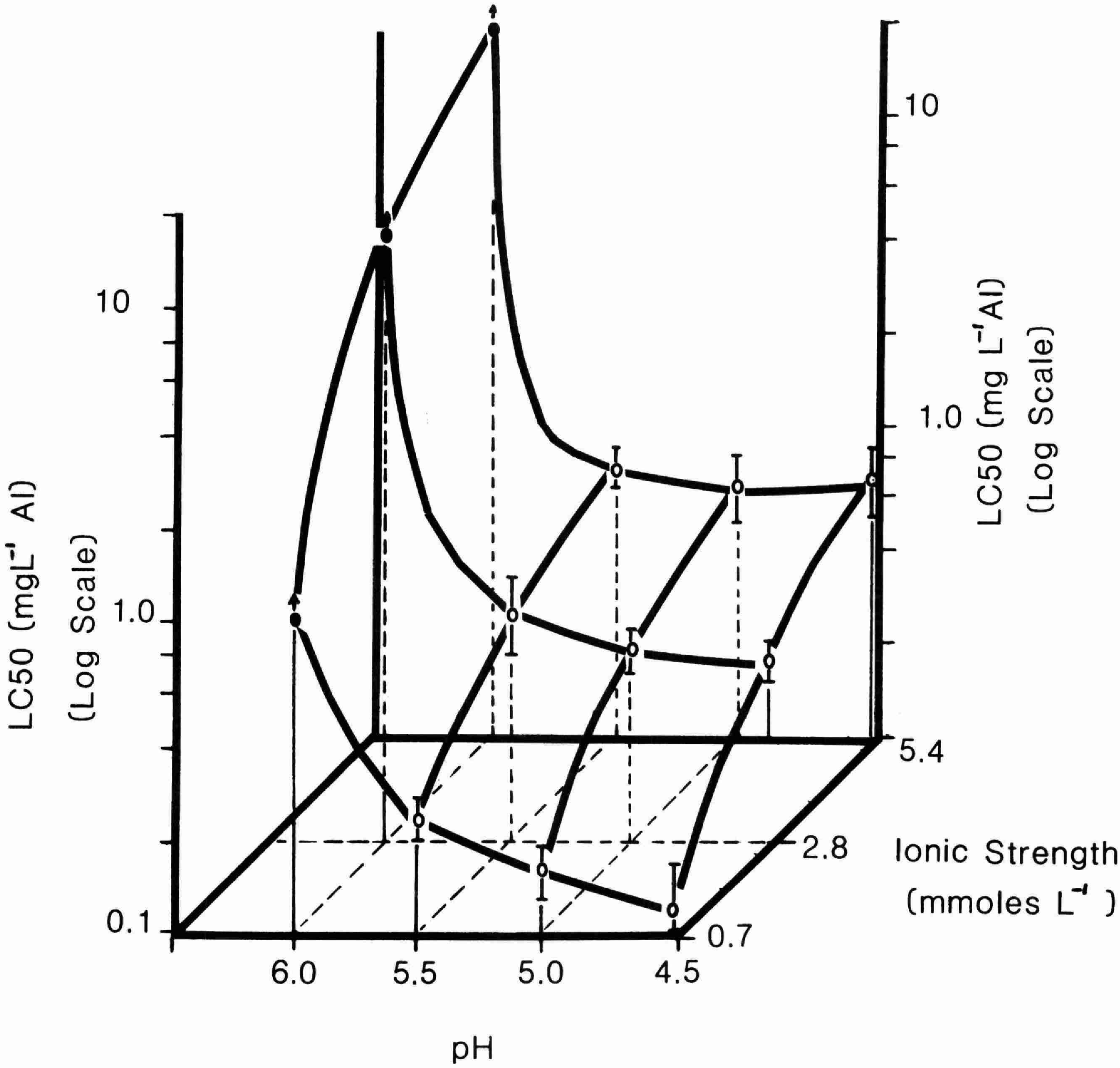
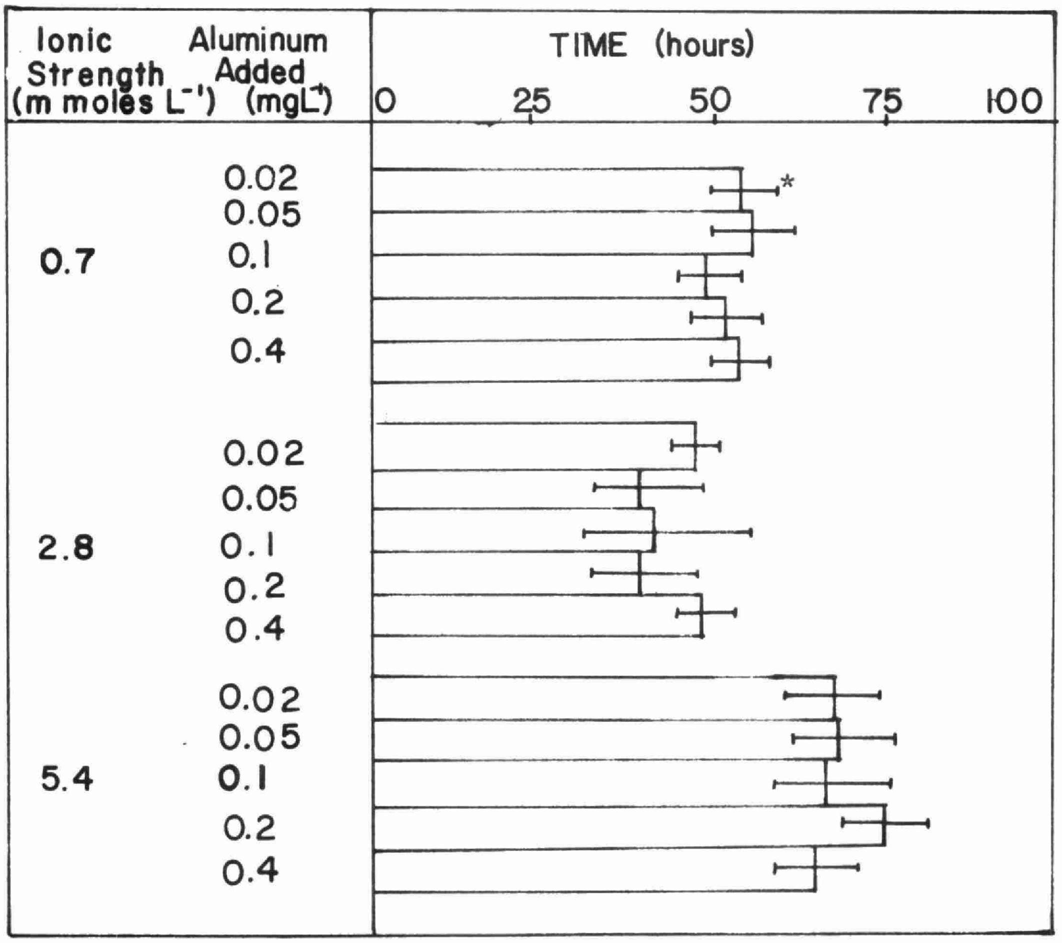


Table 2: Percent Survival of alevin rainbow trout exposed to aluminum and pH at three levels of ionic strength.

pH	Al ($mg\ L^{-1}$)	Ionic Strength Levels ($mmoles\ L^{-1}$) Low (0.73)	Intermediate (2.76)	Tap Water (5.43)
6.0	<0.02	100	100	100
	0.20	100	100	100
	0.40	100	100	100
5.5	<0.02	100	100	100
	0.20	70*	100	100
	0.40	0	100	100
5.0	<0.02	100	100	100
	0.20	30*	100	95
	0.40	0*	60*	90
4.5	<0.02	90	90	85
	0.20	25*	85	95
	0.40	0*	70*	90

*Significant Difference ($p < 0.05$), N = 2.

Fig. 6 Median Survival Times of Alevin Rainbow Trout Subjected to Aluminum at Lethal pH (4.0) in Solutions Varying in Ionic Strength .



*Median survival time (time required to cause 50% mortality of test fish) + 95% Confidence Intervals.

(2) Electrolyte Levels

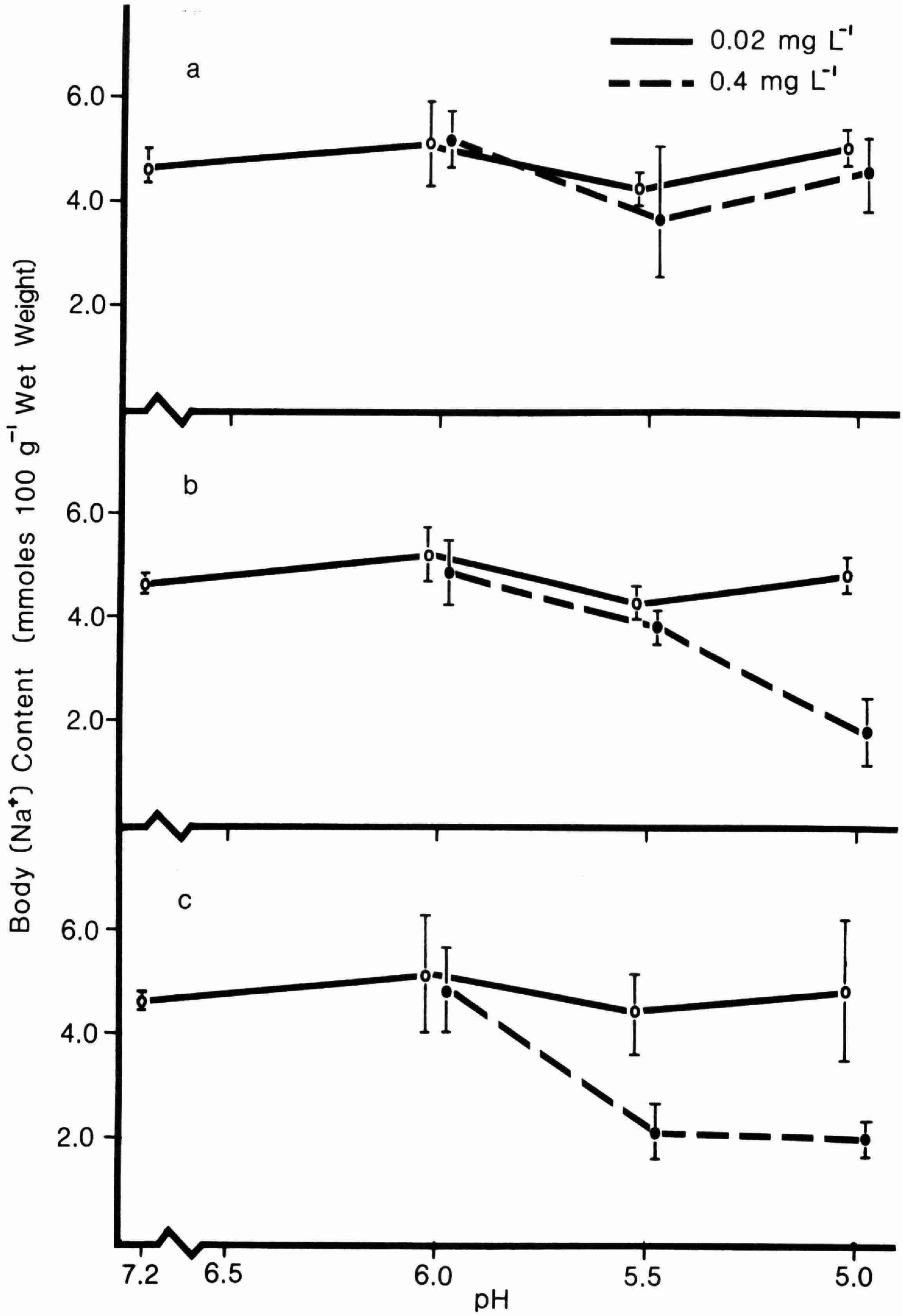
The loss of electrolytes, in particular Na^+ and Cl^-, determined in fish subjected to elevated aluminum (0.4 mg L^{-1}) in solutions ranging in pH (6.0 to 5.0) and ionic strength (5.43 to 0.73 mmoles L^{-1}) closely parallelled the pattern of mortality (Appendix IV-VIII). Alevin rainbow trout maintained normal body content levels of Na^+, K^+, Ca^{++} and Mg^{++} when exposed to low pH (6.0 to 5.0) and aluminum concentration (<0.02 mg L^{-1}) in all waters tested. However, marked reductions in body content levels of Na^+ (Fig. 7) and Cl^- and to a lesser extent K^+, Ca^{++} and Mg^{++} were observed at high aluminum (0.4 mg L^{-1}). The extent of the loss varied with the ion being measured and increased with decreasing ionic strength and pH. Reductions in concentrations of body Na^+ (58.8 to 61.8%), K^+ (16.9 to 20.0%), Ca^{++} (17.4 to 19.6%) and Mg^{++} (18.2 to 20.0%) were greatest at pH 5.0 in low and intermediate ionic strength waters. Ion concentration was not reduced at pH 6.0. The mitigating effects of ionic strength were most evident at pH 5.5. A 50% reduction in body Na^+ and Cl^- content was observed at low ionic strength, while only 10-20% reductions were observed at the intermediate level. Aluminum stress was completely alleviated at the high ionic strength level, as demonstrated by the ability of fish to maintain normal body electrolyte levels under these conditions.

DISCUSSION

Life Stage Sensitivities to Aluminum/pH

Embryos in the cleavage stage were more sensitive to low pH (4.5) than older embryonic and fry stages. Similar results have been noted for brook trout, and white sucker (Baker and Schofield, 1982). Daye and

Fig. 7 Mean Whole Body Sodium (Na^+) Levels in Alevin Rainbow Trout Exposed to Aluminum at Different pH and Ionic Strength Levels



Ionic Strength Level (a) 5.4 mmoles L^{-1} (b) 2.8 mmoles L^{-1} (c) 0.7 mmoles L^{-1}

Garside, (1977), observed that atlantic salmon (Salmo salar) embryos in early cleavage were more sensitive than older encapsulated stages but were less sensitive than fry, suggesting that the greater tolerance of the encapsulated egg was due to the presence of the surrounding perivitelline fluid and zona radiata. The egg membrane, however, did not represent an effective barrier to H^+ ion in experiments conducted with atlantic salmon embryos (Peterson et al. 1980). The pH of the perivitelline fluid was observed to decrease rapidly when embryos were exposed in acid waters (pH 5.5, 4.0).

Reasons for the greater sensitivity of the embryonic stages are not yet known. In older fish, exposure to low pH has been shown to interfere with the regulation of body salts, in particular, Na^+ and Cl^- (Packer and Dunson 1970; Leivestad et al. 1976). Daye and Garside (1980) suggested that the death of pre-hatching embryos exposed to low pH, resulted from injury of the integument which is the principle site of respiration and ion exchange in the embryo (Shen and Leatherland, 1978). Histological examinations of the yolk sac epithelium of rainbow trout embryos in early development, showed fewer numbers of cell types with the capability for ionoregulation than older embryonic stages (Shen and Leatherland, 1978). The greater sensitivity to low pH of the cleavage embryo may in part be related to a more limited capacity to regulate ion levels.

Sensitivity of trout to aluminum on the other hand increased with advancing developmental stages, suggesting that the zona radiata and/or perivitelline fluid associated with the egg stages provided an effective barrier to aluminum ion. Older encapsulated embryos also showed increasing sensitivity to aluminum. Skidmore (1965) observed a similar response in zebrafish embryos (Brachydanio rerio), subjected to zinc. He noted that the egg membrane became increasingly brittle with time suggesting that a change in the integrity of the membrane might also be accompanied by an increasing permeability to metal ions.

Aluminum was most toxic in solutions of low pH (4.5 to 5.5 to all stages except the cleavage embryo. In this case the effects of low pH (4.5) were mitigated by aluminum, possibly in response to the presence of

free aluminum (Al^{3+}). Differences in the relative sensitivities of the various life stages to aluminum were also most pronounced at low pH. Development and survival of the cleavage embryo were unaffected by nominal aluminum concentrations as high as 1.0 mg L^{-1} while survival of the yolk sac and swim up fry stages was significantly reduced ($p < 0.01$) in the presence of 0.5 mg L^{-1} at pH 5.5 and 0.1 mg L^{-1} at pH 4.5. Survival of the eyed embryo was only moderately reduced at high aluminum (1.0 mg L^{-1}) and low pH (4.5 to 5.5) although latent toxic effects of both pH and aluminum were evident during the recovery period.

Influence of Ionic Strength on Aluminum/pH Toxicity

Aluminum toxicity to alevin rainbow trout varied with both pH and ionic strength. Some of this variation is likely due to changes in the relative concentrations of aqueous aluminum species (Driscoll *et al.* 1980). Aluminum was most toxic to trout at low pH (5.0 to 4.5) and ionic strength (0.7 mmoles L^{-1}). Under these conditions, free aluminum, aluminum hydroxide and aluminum fluoride complexes dominated ($Al^{3+} > AlOH^{2+} > AlF^{2+} > Al(OH)_2^+ > AlF_2^+$; pers. comm. Bruce Lazerte). At intermediate and high ionic strength levels, there is a shift to higher order AlF_x complexes due to the higher levels of fluoride in these waters, 0.4 and 0.9 mg L^{-1}, respectively. Significant reductions in aluminum toxicity ($p < 0.05$) were observed in these waters relative to the low ionic strength water suggesting reduced toxicity of the fluoride species. This agrees with the findings of Driscoll *et al.* (1980) who observed a reduction in aluminum toxicity to brook trout exposed at high fluoride level (0.5 mg L^{-1}) relative to the control exposure (<0.01 mg L^{-1}). Aluminum toxicity also decreased with increasing pH ($p < 0.05$), particularly at pH levels >5.5. At the higher pH levels, there is a shift to higher (OH_x) ligands of aluminum, and a general reduction in the total dissolved aluminum because of its decreased solubility. At pH 7.2, the $Al(OH)_4^-$ species appears to dominate. However, no acute toxic effect was associated with this pH.

The relative concentrations of major ions in the test water appeared to have little or no effect on fish response to pH stress. In the

present study, alevins were able to tolerate pH levels as low as 4.5 equally well in solutions ranging in ionic strength (0.7 to 5.4 mmoles L^{-1}) with corresponding calcium and sodium concentrations ranging from 4.2 to 32.5 and 2.9 to 13.1 mg L^{-1} respectively. At lethal pH (4.0) small but significant increases in survival times were observed only at high ionic strength. Packer and Dunson (1970) observed small increases in the survival times of rainbow trout subjected to low pH (3.5) when Na^+ concentrations in the external medium were increased from 100 μM to 150 mM (i.e. a 1500 fold increase in sodium concentration). Leivestad et al., (1980), observed that increasing calcium concentrations in the water usually only delayed the toxic effects of hydrogen ion at lethal pH levels (<4.0). In support of these findings McWilliams et al. (1978), observed that the addition of Ca^{++} to the external medium resulted in a reduction of the gill membrane permeability to Na^+ except in acid solutions ranging in pH from 4.0 to 3.5.

Effects of pH/Aluminum on Electrolyte Levels

Loss of electrolytes has been observed in fish subjected to "severe" acid stress. Packer and Dunson (1970) observed up to 60% reductions in body Na^+ content of rainbow trout exposed to lethal pH (3.5). They suggest that the loss of Na^+ occurred in response to greatly increased Na^+ efflux rate and to a lesser extent, to a decreased rate of Na^+ influx. In laboratory and field studies Leivestad and Muniz (1976) noted decreased levels of plasma Na^+ and Cl^- in brown trout severely stressed by low pH (4.0). More recent studies (Muniz and Leivestad, 1980), revealed similar effects in brown trout subjected to low but sublethal pH (5.0-5.5) in the presence of elevated aluminum concentrations. Similarities in the nature and extent of the response to lethal (pH <4.0) versus sublethal pH/elevated aluminum was seen as suggestive that the fish gill, which appears to be the primary target site, is unable to distinguish between the two stress factors.

Results of the present study demonstrated that whole body content levels of inorganic ions decreased in response to aluminum at sublethal

pH levels. Death of alevin rainbow trout subjected to elevated aluminum in acid solutions (pH 5.5 to 5.0) was preceeded by marked reductions in whole body Na^+ and Cl^- while lower but significant ($p < 0.05$) reductions in K^+, Ca^{++} and Mg^{++} were also observed. The ion loss was greatest at pH 5.0 at low and intermediate ionic strength levels. Alevins however, showed no observable signs of stress at pH values as low as 5.0 in the absence of aluminum (<0.02 mg L^{-1}) and were capable of maintaining normal electrolyte levels.

Relevance

The results of this study demonstrate that fish tolerances to low pH and elevated aluminum differ with life stage exposed and water quality conditions (ionic strength). Yolk sac and swim up fry are more sensitive to aluminum in combination with low pH than other life stages, while eggs are more sensitive to pH stress alone. These stages are particularly sensitive to acid stressed waters of low ionic strength (<0.7 mmoles L^{-1}) and elevated aluminum (0.1 to 0.2 mg L^{-1}) levels, similar to those observed in some stream and lake littoral zones in the Muskoka-Haliburton area.

In the labortory, mortalities of egg and fry stages, in response to artificially induced stress levels are relatively easy to measure. However, in the field, fish mortalities are generally not detected, particularly at the time of occurrence and may not be apparent unless extensive fisheries programs are undertaken which identify year class structures. Consequently this study provides insight into a possible mechanism of recruitment failure. In addition, this information may ultimately be used in combination with water chemistry data for the development of predictive fisheries models.

SUMMARY

(1) Sensitivity of rainbow trout to hydrogen ion decreased with increasing level of development (cleavage egg > eyed egg > yolk sac fry ≃ swim up fry).

(2) Sensitivity to aluminum varied with pH, ionic strength and developmental stage.

(a) Aluminum was generally more toxic in solutions of low pH (5.5 to 4.5) and decreasing ionic strength (5.43 to 0.73 mmoles L^{-1})

(b) Trout were most sensitive to aluminum during the yolk sac and swim up fry stages and least sensitive to aluminum during the cleavage stage.

(c) Aluminum was beneficial to the survival of cleavage embryos at pH 4.5.

(3) Continuous acidification of surface waters in the Muskoka-Haliburton area may pose a serious threat to the existing fisheries. As shown in this work, the combination of aluminum and low pH is deleterious to the survival of important life stages of fish.

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APPENDIX I: Chemical characteristics of low, intermediate and high ionic strength waters.

Parameter ($mg\ L^{-1}$)	Ionic Strength					
	Low		Intermediate		High	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Hardness[1]	14.3	1.38	58.9	4.02	113.8	6.71
Ca	4.2	0.37	17.1	1.02	32.5	2.46
Mg	0.9	0.15	3.8	0.20	7.8	0.24
Na	2.9	0.18	7.0	0.30	13.4	0.93
K	0.7	0.11	1.0	0.12	1.6	0.09
Cl	4.8	0.20	15.6	0.85	30.5	0.50
Fl	0.1	0.01	0.4	0.03	0.9	0.04
DOC	0.4	0.25	0.7	0.15	1.6	0.08
DIC	0.9	0.32	0.8	0.39	0.8	0.52
NH_3	0.118	0.0584	0.096	0.0561	0.091	0.0272
NO_2	0.002	0.0013	0.002	0.0013	0.002	0.0013
NO_3	0.16	0.0177	0.271	0.0366	0.416	0.0527
Cu	0.002	0.0006	0.003	0.0010	0.004	0.0006
Ni	0.002	0.0006	0.004	0.0006	0.005	0.0006
Zn	0.008	0.0042	0.012	0.0029	0.015	0.0023
Cd	<0.001	0.0002	<0.001	0.0001	<0.001	0.0001
Co	0.001	0.0002	0.003	0.0010	0.003	0.0010
Cr	0.001	0.0003	0.001	0.0006	0.002	0.0006
Pb	0.006	0.0025	0.012	0.0040	0.007	0.0010
Fe	0.017	0.0115	0.017	0.0115	0.013	0.0058
Mn	0.003	0.0012	0.003	0.0012	0.002	0.0058

[1] $mg\ L^{-1}$ as $CaCO_3$

APPENDIX IIa: Summary of the nominal, measured and theoretical, aluminum concentrations in the test waters: Low Ionic Strength (0.7 mmoles L^{-1}).

pH	Nominal Aluminum (mg L^{-1})	Total Measured Aluminum (mg L^{-1}) After 0 hr	96 hr	Theoretical Dissolved Inorganic Aluminum (mg L^{-1})
7.2	.020	.020	.020	.020
	.500	.330	.110	.204
	1.000	.750	.200	.204
6.5	.020	.020	.020	.020
	.100	.110	.050	.047
	.500	.320	.070	.048
	1.000	.760	.220	.048
5.5	.020	.020	.020	.020
	.100	.080	.050	.100
	.500	.410	.210	.153
	1.000	.850	.500	.157
4.5	.020	.020	.020	.020
	.100	.110	.080	.100
	.500	.410	.440	.500
	1.000	.860	.840	.877

0 and 96 hr measured aluminum concentrations are average values, N = 8.

APPENDIX IIb: Summary of the nominal, measured and theoretical aluminum concentrations in the test waters: Low Ionic Strength (0.7 m moles L^{-1}).

pH	Nominal Aluminum (mg L^{-1})	Total Measured Aluminum (mg L^{-1}) After 0 hr	96 hr	Theoretical Dissolved Inorganic Aluminum (mg L^{-1})
6.0	.020	N.A.	.020	.020
	.050	N.A.	.090	.050
	.100	N.A.	.100	.065
	.200	N.A.	.090	.065
	.400	N.A.	.200	.065
5.5	.020	.020	.020	.020
	.050	-	.060	.050
	.100	.070	.080	.100
	.200	.150	.070	.149
	.400	.310	.170	.151
5.0	.020	.020	.020	.020
	.050	.040	.040	.050
	.100	.070	.040	.100
	.200	.170	.040	.200
	.400	.330	.210	.260
4.5	.020	.020	.020	.020
	.050	.050	.070	.050
	.100	.100	.120	.100
	.200	.220	.220	.200
	.400	.400	.410	.400
4.0	.020	.020	N.A.	.020
	.050	.060	N.A.	.050
	.100	.100	N.A.	.100
	.250	.280	N.A.	.250
	.500	.530	N.A.	.500

0 and 96 hr measured aluminum concentrations are from a single sample.

APPENDIX IIc: Summary of the nominal, measured and theoretical aluminum concentrations in the test waters: Intermediate Ionic Strength (2.8 mmoles L^{-1}).

pH	Nominal Aluminum (mg L^{-1})	Total Measured Aluminum (mg L^{-1}) After 0 hr	96 hr	Theoretical Dissolved Inorganic Aluminum (mg L^{-1})
6.0	.020	N.A.	.020	.020
	.050	N.A.	.080	.050
	.100	N.A.	.140	.100
	.200	N.A.	.240	.200
	.400	N.A.	.310	.213
5.5	.020	.020	.020	.020
	.050	.060	.070	.050
	.100	.110	.060	.100
	.200	.190	.120	.200
	.400	.250	.290	.400
5.0	.020	.020	.020	.020
	.050	.060	.040	.050
	.100	.090	.050	.100
	.200	.220	.150	.200
	.400	.250	.290	.400
4.5	.020	.020	.020	.020
	.050	.050	.110	.050
	.100	.160	.170	.100
	.200	.220	.150	.200
	.400	.430	.370	.400
4.0	.020	.020	N.A.	.020
	.050	.060	N.A.	.050
	.100	.100	N.A.	.100
	.250	.260	N.A.	.250
	.500	.520	N.A.	.500

0 and 96 hr measured aluminum concentrations are from a single sample.

APPENDIX IId: Summary of the nominal, measured and theoretical aluminum concentrations in the test waters: High Ionic Strength (5.4 mmoles L^{-1}).

pH	Nominal Aluminum (mg L^{-1})	Total Measured Aluminum (mg L^{-1}) After 0 hr	96 hr	Theoretical Dissolved Inorganic Aluminum (mg L^{-1})
6.0	.020	N.A.	.020	.020
	.050	N.A.	.050	.050
	.100	N.A.	.110	.100
	.200	N.A.	.220	.200
	.400	N.A.	.380	.400
5.5	.020	.020	.020	.020
	.050	.110	.080	.050
	.100	.100	.100	.100
	.200	.210	.160	.200
	.400	.330	.280	.400
5.0	.020	.020	.020	.020
	.050	.070	.040	.050
	.100	.160	.060	.100
	.200	.170	.250	.200
	.400	.430	.350	.400
4.5	.020	.020	.020	.020
	.050	.070	.060	.050
	.100	.180	.120	.100
	.200	.280	.210	.200
	.400	.480	.420	.400
4.0	.020	.020	N.A.	.020
	.050	.090	N.A.	.050
	.100	.170	N.A.	.100
	.250	.300	N.A.	.250
	.500	.500	N.A.	.500

0 and 96 hr measured aluminum concentrations are from a single sample.

APPENDIX III: Body content levels of Na^+, Cl^-, K^+, Ca^{++} and Mg^{++} (mmoles 100 g^{-1} wet weight) determined in alevin rainbow trout acclimated for 60 days to solutions of different ionic strength.

Inorganic Ion		Ionic Strength (mmoles L^{-1})					
		Low (0.7)		Intermediate (2.8)		Tap Water (5.4)	
	N	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Na^+	9	4.59	0.106	4.64	0.070	4.78	0.305
Cl^-	3	3.90	0.29	4.02	0.070	4.0	0.08
K^+	9	7.94	0.386	8.16	0.568	7.82	0.660
Ca^{++}	9	9.46	1.309	9.28	1.320	8.49	0.909
Mg^{++}	9	0.97*	0.041	1.00*	0.080	0.87	0.086

* = Significant difference ($p = 0.01$) from high ionic strength level (5.4 mmoles L^{-1})

APPENDIX IV: Mean whole body Na^+ levels (mmoles $100g^{-1}$ wet wt.) in rainbow trout alevins exposed to combinations of aluminum and pH in waters of different ionic strength.

pH	Al ($mg\ L^{-1}$)	Ionic Strength ($mmoles\ L^{-1}$) Low (0.7) $\bar{x}$	S.D.	Intermediate (2.8) $\bar{x}$	S.D.	Tap Water (5.4) $\bar{x}$	S.D.
7.2	<0.02	4.49	0.126	4.71	0.331	4.64	0.252
6.0	<0.02	5.15	0.452	5.08	0.331	5.14	0.331
6.0	0.40	4.86	0.331	4.86	0.252	5.22	0.217
7.2	<0.02	4.57	0.574	4.57	0.218	4.57	0.218
5.5	<0.02	4.42	0.331	4.28	0.126	4.28	0.126
5.5	0.40	2.21*	0.213	3.84*	0.126	3.70*	0.574
7.2	<0.02	4.70	0.305	4.64	0.126	5.13	1.196
5.0	<0.02	4.93	0.665	4.79	0.218	5.08	0.126
5.0	0.40	2.03*	0.052	1.83*	0.270	4.64	0.331

*Significant difference ($p < 0.01$), N = 3.

APPENDIX V: Mean whole body Cl^- levels (mmoles $100g^{-1}$ wet wt.) in alevin rainbow trout exposed to combinations of aluminum and pH in waters of different ionic strength.

pH	Al (mg L^{-1})	Ionic Strength (mmoles L^{-1}) Low (0.3) $\bar{x}$	S.D.	Intermediate (2.8) $\bar{x}$	S.D.	Tap Water (5.4) $\bar{x}$	S.D.
7.2	<0.02	3.09	0.29	4.02	0.07	4.0	0.08
5.5	<0.02	4.14	0.43	3.95	0.14	4.04	0.08
5.5	0.40	2.07*	0.19	3.20*	0.16	3.24*	0.61

*Significant difference ($p < 0.01$), N = 3.

APPENDIX VI: Mean whole body K^+ levels (mmoles $100g^{-1}$ wet weight) in alevin rainbow trout exposed to combinations of aluminum and pH in waters of different ionic strength.

pH	Al (mg L^{-1})	Ionic Strength (mmoles L^{-1}) Low (0.7) $\bar{x}$	Low (0.7) S.D.	Intermediate (2.8) $\bar{x}$	Intermediate (2.8) S.D.	Tap Water (5.4) $\bar{x}$	Tap Water (5.4) S.D.
7.2	<0.02	8.11	0.19	8.19	0.338	8.19	0.128
6.0	<0.02	8.62	0.19	8.53	0.074	8.28	0.074
6.0	0.40	8.11	0.074	8.49	0.266	8.19	0.256
7.2	<0.02	7.47	0.412	7.55	0.256	7.12	0.19
5.5	<0.02	7.25	0.451	7.42	0.128	6.96	0.074
5.5	0.40	6.51*	0.374	6.91*	0.256	7.04	0.266
7.2	<0.02	8.50	0.410	8.75	0.19	8.11	0.704
5.0	<0.02	8.32	0.443	8.53	0.266	8.06	0.558
5.0	0.40	6.88*	0.257	6.83*	0.604	7.89	0.369

*Significant difference ($p < 0.01$), N = 3.

APPENDIX VII: Mean whole body Ca^{++} levels (mmoles $100g^{-1}$ wet weight) in alevin rainbow trout exposed to combinations of aluminum and pH in waters of different ionic strength.

pH	Al (mg L^{-1})	Ionic Strength (mmoles L^{-1}) Low (0.7) $\bar{x}$	S.D.	Intermediate (2.8) $\bar{x}$	S.D.	Tap Water (5.4) $\bar{x}$	S.D.
7.2	<0.02	9.0	1.54	9.33	0.81	7.67	0.07
6.0	<0.02	10.36	0.37	9.25	0.43	8.0	0.65
6.0	0.40	8.62	0.57	9.5	0.87	8.17	0.19
7.2	<0.02	11.29	0.38	10.63	0.78	9.38	0.57
5.5	<0.02	11.38	0.33	10.83	0.40	10.33	0.32
5.5	0.40	10.40	0.65	10.5	0.22	9.21	0.85
7.2	<0.02	8.17	0.49	7.92	0.19	8.42	0.88
5.0	<0.02	8.5	0.63	8.17	0.19	7.58	0.71
5.0	0.40	6.83*	0.19	6.75*	0.13	7.67	0.40

*Significant difference ($p < 0.01$), N = 3.

APPENDIX VIII: Mean whole body Mg^{++} levels (mmoles 100 g^{-1} wet weight) in alevin rainbow trout exposed to combinations of aluminum and pH in waters of different ionic strength.

		Ionic Strength (mmoles L^{-1})					
pH	Al (mg L^{-1})	Low (0.7)		Intermediate (2.8)		Tap Water (5.4)	
		$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
7.2	<0.02	0.96	0.025	0.95	0.041	0.81	0.025
6.0	<0.02	0.99	0.041	0.95	0.041	0.81	0.049
6.0	0.40	0.95	0.041	1.00	0.025	0.88	0.025
7.2	<0.02	1.02	0.025	0.98	0.062	0.88	0.049
5.5	<0.02	0.96	0.119	1.00	0.049	0.92	0.103
5.5	0.40	0.89	0.091	0.96	0.025	0.85	0.025
7.2	<0.02	1.03	0.053	1.10	0.025	0.92	0.132
5.0	<0.02	1.04	0.025	1.03	0.041	0.93	0.086
5.0	0.40	0.85	0.025	0.82*	0.070	0.89	0.062

*Significant difference ($p < 0.05$), N = 3.